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PCT

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<p>(51) International Patent Classification⁵ : A61K 31/215, 31/275, 31/235 A61K 31/24, 31/19, 31/195 // A61K 31/17, 31/135, 31/13 A61K 31/12, 31/115, 31/095 A61K 31/075, 31/045, 31/05 A61K 31/03, 31/025, 31/015</p>	<p>A1</p>	<p>(11) International Publication Number: WO 92/18119</p> <p>(43) International Publication Date: 29 October 1992 (29.10.92)</p>
<p>(21) International Application Number: PCT/US92/03209</p> <p>(22) International Filing Date: 16 April 1992 (16.04.92)</p> <p>(30) Priority data: 687,719 18 April 1991 (18.04.91) US</p> <p>(71) Applicant: WORLD RESEARCH INSTITUTE FOR SCIENCE AND TECHNOLOGY, INC. [US/US]; 38-42 9th Street, Long Island City, NY 11101 (US).</p> <p>(72) Inventors: BANG, Soon, Duk ; 13859 Stonebrook Court, Clifton, VA 22024 (US). JOHNSON, Stuart, K. ; 147-12 84th Avenue, Briarwood, NY 11435 (US). PARK, John, C., S. ; 33 Iroquois Avenue, Oakland, NJ 07436 (US).</p>		<p>(74) Agents: BARRESE, Rocco, S. et al.; 333 Earle Ovington Blvd., Uniondale, NY 11553 (US).</p> <p>(81) Designated States: AT (European patent), AU, BE (Euro- pean patent), BR, CA, CH (European patent), DE (Eu- ropean patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), MC (European patent), NL (Eu- ropean patent), RU, SE (European patent).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: COMPOSITION AND METHOD FOR TREATING TUMORS</p> <p>(57) Abstract</p> <p>A composition and method for treating tumors with resin acids and derivatives thereof are provided.</p>		

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COMPOSITION AND METHOD FOR TREATING TUMORS

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BACKGROUND OF THE INVENTION

5 The present invention is directed to a composition and method for treating tumors. More specifically, the present invention is directed to the treatment of tumors involving administration of certain rosin extracts including abietic acid, other resin acids, and their derivatives.

10 Traditionally, tumors have been treated by surgery, radiation, chemotherapy, or a combination of any of these treatments. Recognition that malignant cells tend to spread systemically through the body has resulted in recent emphasis on chemotherapeutic treatment to attack such tumor cells. However, chemotherapy administration is, at the very
15 least, extremely debilitating, while toxicity continues to be a difficult problem. Additionally, difficulties have been encountered with selectivity of action by chemotherapeutic agents against certain malignant cells, while problems of providing a suitable delivery mechanism of
20 such drugs have also been experienced. Accordingly, the search continues for improved anti-cancer treatments which minimize toxicity, improve selectivity of action (i.e. attack only malignant tumors), and enhance delivery of active agents to the situs of such malignant tumors and
25 cells.

For example, U.S. Patent No. 4,193,931 discloses 7
-(substituted indanyl or naphthyl)-3-methyl-octa-2,4,6-
triene derivatives which are useful as anti-tumor agents.
U.S. Patent No. 4,786,496 relates to an immunopotentiator
30 having anti-tumor activity which is derived from marine
chlorella. Ref. Zh. Khim 1971, Abstr. No. 8Zh623 (CA76:

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1 141080g (1972)) states that certain specific alkylating
 derivatives of abietic acid, dehydroabietic acid and 6-
 hydroxyabietic acid exhibited antitumor activity against
 certain sarcomas. At the same time, other alkylating
 5 derivatives of these acids were found to be very toxic,
 while still other alkylating derivatives were documented as
 exhibiting no or very weak anti-tumor activity.

Pharmaceutical Chemistry Journal Volume 6, No. 10 (1972),
 pages 647-650 also documents these specific test results.

10

SUMMARY OF THE INVENTION

It is an object of the present invention to
 provide a composition and method for the treatment of
 tumors.

15 It is a particular object of the invention to
 provide a tumor-treating composition of reduced toxicity.

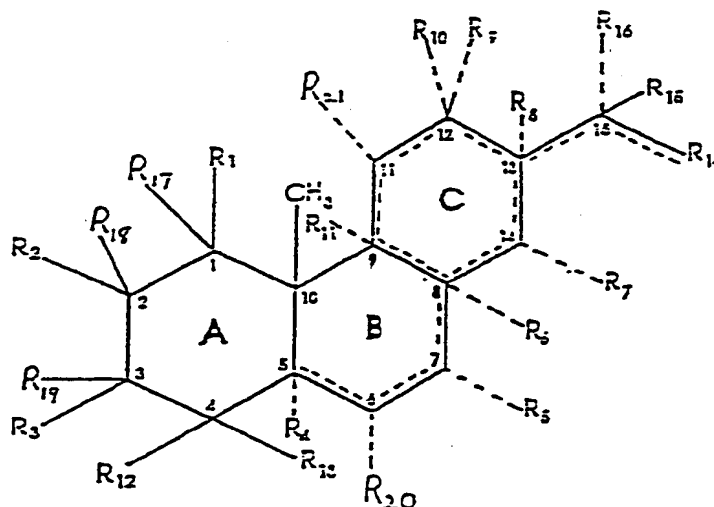
It is another object of the present invention to
 provide for improved selectivity in treating tumors.

20 These and other objects are achieved by the tumor-
 treating composition of the present invention which
 comprises a tumor-treating effective amount of at least one
 compound of the general formula (I):

25

30

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(I)

- 1 wh rein R_1 is H or OH,
 R_2 is H, OH or OCOOH,
 R_3 is H, OCH₃ or CH(COOCH₃)₂,
 R_4 , when present, is H or OCH₃,
5 R_5 , when present, is H, =O, CH₂OH or CH₂OCOCH₃,
 R_6 , when present, is H, OH, CHO or CHS₂,
 R_7 , when present, is H, =O, Cl, OH, OCH₃, COOH,
OCOOH, CH₂OH or CH₂OCOCH₃,
 R_8 , when present, is H/^{CH₃}OH,
10 R_9 , when present, is H or CH₃,
 R_{10} , when present, is H, Cl, CH₃, CH₂Cl, CH₂CN,
CH₂OH, COOCH₃, CH₂COOH, CH₂CH₂NH₂, CH₂OCOCH₃,
CH₂CH₂NCO, CH₂COOCH₃, CH₂CH₂NHCONHC₆H₅,
CH₂O(CH₂CCH₃OH)_xH where x is a positive
15 integer, CH₂CH₂NHCONHC₆H₁₁, CH₂OCO(CH₂)₄COOC₂H₄OH
or CH₂CH₂NH₂·HOOC₆H₃(NO₂)₂,
 R_{11} , when present, is H or OH,
 R_{12} is H, CH₃ or COOCH₃,
 R_{13} is CN, CH₃, COCl, COOH, CONH₂, COCH₃, CH₂OH,
20 CH₂NH₂, COOCH₃, CH₂NCO, CH₂OCOOH, CH₂OCOCH₃,
CH₂O(CHCHO)_xH where x is a positive integer,
CH₂NHCONHC₆H₅, CH₂NHCONHC₆H₁₁, CH₂O
(CH₂CCH₃HO)_xH where x is a positive integer,
CH₂NH₂·HOCC₆H₃(NO₂)₂ or
25 CH₂OCO(CH₂)₄COOC₂H₄OH,
 R_{14} is H, CH₃, CH₂, COOH, CH₂OH or COOCH₃,
 R_{15} is H, OH, OCOOH, ^{H,}
 R_{16} , when present, is /CH₃ or CH₂OH,
 R_{17} is H or OH,
30 R_{18} is H, OH or OCOOH,

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- 1 R_{19} is H, OCH_3 or $CH(COOCH_3)_2$,
 R_{20} , when present, is H, =O, CH_2OH or CH_2OCOCH_3 ,
 and
 R_{21} , when present, is H or CH_3 ,
5 and the pharmaceutically acceptable salts thereof
together with a pharmaceutically acceptable carrier
therefor.

 In formula (I) supra, the dashed lines (-----)
denote optional presence of substituents and optional double
10 bonds, i.e., unsaturation. For example, unsaturation of
bonds can be present at any of the positions on the "C" ring
in formula (I) supra, e.g., position nos. 8, 9, 11, 12, 13
and 14. If, e.g., unsaturation is observed at the no. 12
position on ring "C" in formula (I) supra, namely a double
15 bond is present either from position no. 12 to position no.
11 or from position no. 12 to position no. 13, then one of
groups R_9 and R_{10} will not be present. However, there is no
requirement that unsaturation must be present at any of the
positions on rings B or C or along the chain extending from
20 the no. 13 position on ring "C".

 The present invention is also directed to a method
for treating a tumor which comprises administering a tumor-
treating effective amount of a compound of the above formula
(I) to a mammal possessing a tumor.

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BRIEF DESCRIPTION OF THE DRAWINGS

 The present invention will be explained in greater
detail with reference to the accompanying drawings in which:

 Fig. 1 is a graph comparing retention time of
30 different rosin fractions, including those of the present

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1 invention, eluted from a normal phase chromatographic
column, with ultraviolet light absorbance and inhibition of
tumor cell growth;

Fig. 2 is a graph comparing retention time of a
5 purified resin acid fraction from Fig. 1 eluted from a
reverse phase chromatographic column, with ultraviolet light
absorbance and inhibition of tumor cell growth;

Fig. 3 is a graph comparing survival times of mice
treated with different compositions, including those of the
10 present invention; and

Fig. 4 is a graph comparing weight gain of the
mice treated with the compositions of Fig. 3.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

15 The active tumor-treating agents of this invention
are all rosin extracts including abietic acid, other resin
acids, and derivatives thereof. Resin acids including
abietic acid are isolated from rosin which is obtained
primarily from coniferous trees. Rosin is a complex mixture
20 of compounds with abietic acid being a principal constituent
thereof. Rosin is easily modified to yield a number of
different products, including levopimaric and abietic acids.
In particular, abietic acid is prepared by isomerization of
rosin as presented in Harris, Sanderson, Org. Syn. Coll.
25 Vol. IV, 1 (1963). For example, abietic acid can be
prepared by heating rosin alone or with other acids (Merck
Index, Tenth Edition, Entry #1).

More specifically, the limpid oleoresin exuding
from incisions cut in the bark of living pine trees is
30 separated by steam distillation into a steam volatile
fraction, a small amount of gum turpentine, and a

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1 nonvolatile residue which, upon cooling, forms a
yellow/brown glassy substance which is known as rosin.
Rosin is primarily composed of diterpene acids of the
formula $C_{19}H_{29}COOH$ in various stages of isomerization which
5 are known as resin acids (Fieser and Fieser, "Natural
Products Related to Phenanthrene", Third Edition, 1949,
Reinhold Publishing Corporation (New York)). Other
extractable acids from pine are predominantly fatty acids.
Virtually all pine resin acids belong to one of four basic
10 ring structures, abietane, pimarane, isopimarane and
labdane. Some of these resin acids undergo isomerization
and disproportionation upon exposure to inorganic acid
and/or heat. For example, levopimaric acid which is also a
principal resin acid component from coniferous trees is
15 transformed in early stages of heating to roughly equal
amounts of palustric and abietic acids, followed by further
isomerization of palustric acid into abietic acid, as noted
in J. Am. Chem. Soc. 77:2823-2825.

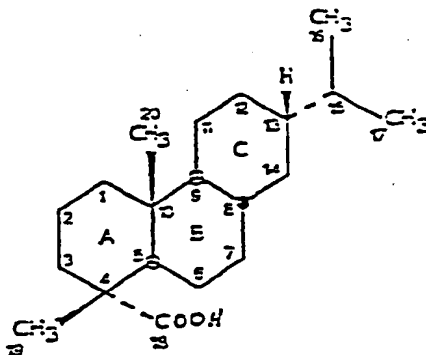
The abietic acid, which is the end product of acid
20 isomerization of pine tree extract, is partially isomerized
to neoabietic acid upon further heating, while the abietic
acid suffers disproportionation at higher temperature to
yield mixtures of dehydroabietic acid, dihydroabietic acid,
and tetrahydroabietic acid (Fieser and Fieser, supra).
25 Preferably, abietic acid can be purified from crude resin by
High Performance Liquid Chromatography (HPLC) which involves
pumping the resin through a column packed with substrate,
whereby the resin fractionates into various constituents,
including the abietic acid, as the resin flows along the
30 column. The resulting abietic acid fraction is then eluted

1 at the appropriate rate from the column and preferably
pass d through a second column packed with different
substrat to further purify the acid, followed by elution
once again.

5 The purified abietic acid fraction can be
esterified by the procedure described in J. Lipid Res., 5:
600-608 to form an abietic acid derivative in formula (I)
supra where R_{13} is COOCH_3 . In this procedure, the abietic
acid fraction and respective alcohol are dissolved in a
10 suitable organic solvent such as hexane, and then heated in
a water bath, preferably from about 95 to about 100°C for
about 20 to about 60 minutes to generate the compound of
formula (I) supra where R_{13} is COOCH_3 . Water is then added
to form two separate layers, namely an aqueous layer and an
15 organic layer, with the organic layer being separated and
evaporated to dryness. The resulting acid ester is then
purified by the HPLC procedure described supra.

Three main classes of resin acids, derived from
the abietane, pimarane and isopimarane skeletons, were
20 isolated from rosin. Formulas (II), (III) and (IV)
illustrate these classes, shown as the fully saturated resin
acid.

18 - ABIETANOIC ACID

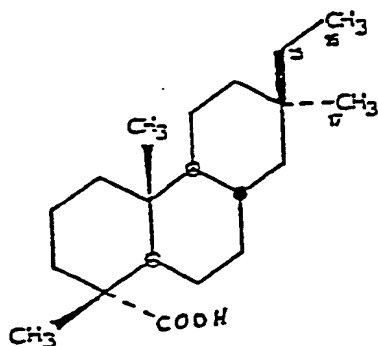


(II)

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18 - PIMARANOIC ACID

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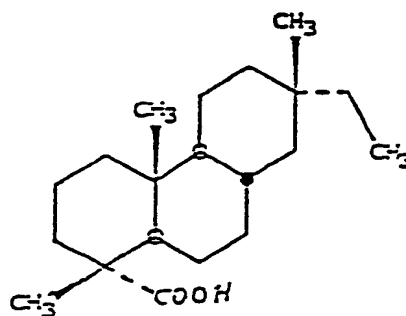


(III)

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18 - ISOPIMARANOIC ACID

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(IV)

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In formulas (II), (III) and (IV) supra, the dashed lines (-----) denote positioning of the substituent groups below the plane and the flared lines denote positioning of the substituent groups above the plane. By the same token, the hollow or open circles on the ring structure denote positioning of the respective juncture below the plane,

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while the filled in circle denotes positioning of the

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1 respective juncture above the plane, in formulas (II), (III)
and (IV). In other words, all conceivable isomers of
abietic, isopimaric, and pimaric acids are encompassed by
the resin acids of the present invention. By the same
5 token, all isomers of any derivatives of these resin acids
(where the R groups represent any of the other substituents
noted in formula (I) supra) are encompassed by the present
invention.

More particularly, the derivatives of abietic,
10 isopimaric and pimaric acids can be prepared by procedures
conventionally available in the field. For example, an
abietic acid derivative of formula (I) supra where R_{10} is
 CH_2OH , $CH_2O(CH_2CCH_3OH)_xH$, $CH_2OCO(CH_2)_4COOC_2H_4OH$, R_{13} is $COOH$,
 CH_2OH , $CH_2O(CH_2CCH_3OH)_xH$, $CH_2OCO(CH_2)_4COOC_2H_4OH$, and unsaturated
15 bonding can be present between the no. 7 and no. 8
positions, the no. 8 and no. 14 positions, and the no. 14
and no. 13 positions on the ring structure, e.g., 12-
hydroxymethyldihydroabietic acid and the dihydro isomers
thereof, can be prepared by the procedure outlined in Ind.
20 Eng. Chem. Prod. Res. Develop. Vol. 9, No. 3 (1970): 304-
310. An abietic acid derivative of formula (I) supra where
 R_5 is H or $=O$, R_{12} is $COOCH_3$, and R_{13} is $COOH$ or $COOCH_3$, and
unsaturation is observed around the "C" ring, i.e. the "C"
ring is aromatic, can be prepared by the procedure outlined
25 in Synthetic Communications 6 (8): 559-561 (1976). An
abietic acid derivative of formula (I) supra where R_{13} is
 $COOH$ or $COOCH_3$, R_5 is H or CH_2OH , R_7 is H or CH_2OH , R_{10} is H or
 CH_2OH , and there are double bonds between the nos. 7 and 8
positions and between the nos. 13 and 14 positions on the
30 ring structure, can be prepared according to the procedure

- 1 described in Ind. Eng. Chem. Prod. Res. Develop. Vol. 12,
No. 3 (1973): 241-245.

An abietic acid derivative of formula (I) supra
where R_{13} is COOH or COOCH₃, R_{10} is CH₂OH, and in which double
5 bonds are present between the nos. 7 and 8 positions, and
optionally between the nos. 9 and 11 positions on the ring
structure, can be prepared according to the procedure
described in J. Org. Chem. 34 (1968): 1940-1942, while
synthesis of this compound is also described in J. Org.
10 Chem. 32 (1967): 3758-3762; this latter reference also
describes synthesis of an abietic acid derivative of formula
(I) supra where R_{13} is COOH or COOCH₃, R_{10} is CH₂OH, CH₂OCOCH₃,
or CH₃, and R_{11} is H or OH.

Preparation of abietic acid derivatives of formula
15 (I) above where R_{13} is COOH, COOCH₃ or CH₂OCOCH₃, R_5 is CH₂OH
or CH₂OCOCH₃, R_{10} is CH₂OCOCH₃, CH₂OH, CH₃ or H, and a double
bond is present between the nos. 7 and 8 positions, and/or
between the nos. 8 and 14 positions, and/or between the nos.
13 and 14 positions on the ring structure, is described in
20 J. Org. Chem. 32: 3763-3767 (1967). Synthesis of various
dinitrile, diamine and diisocyanate derivatives of
hydroxymethylabietanoic acid, namely the compound of formula
(I) supra where R_{13} is COOH, COOCH₃, COCl, CONH₂, CN, CH₂NH₂,
CH₂NHCONHC₆H₅, CH₂NH₂·HO₂CC₆H₃(NO₂)₂, CH₂NCO or CH₂NHCONHC₆H₁₁, and
25 R_{10} is CH₂OH, CH₂OCOCH₃, CH₂Cl, CH₂CN, CH₂COOH, CH₂COOCH₃,
CH₂CH₂NH₂, CH₂CH₂NHCONHC₆H₅, CH₂CH₂NH₂·HO₂CC₆H₃(NO₂)₂, CH₂CH₂NCO, or
CH₂CH₂NHCONHC₆H₁₁ is set forth in J. Chem. Eng. Data Vol. 16,
No. 13 (1971): 299-301.

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1 J. Org. Chem. Vol. 36, No. 26 (1971): 3899-3906
describes synthesis of abietic acid derivatives of formula
(I) above where R_{13} is COOH or COOCH₃, R_{10} is H, COOH or
COOCH₃, R_8 , when present, is OH, and R_7 is =O, OCH₃ or OH.
5 Additionally, J. Org. Chem. Vol. 36, No. 22 (1971): 3271 -
3276 discloses the synthesis of abietic derivatives of
formula (I) supra where R_{13} is COOH and double bonds are
present between the nos. 8 and 14 positions on ring "C" and
also between the no. 13 position on ring "C" and the no. 15
10 carbon atom. Lower life forms such as microbes can be
utilized to synthesize several of the resin acid derivatives
of formula (I) supra, as disclosed in Acta Chemica
Scandinavica B 33 (1979): 76-78, Helvetica Chemica Acta -
Vol. 65, Fasc. 5 (1982) - Nr-127: 1343-1350, and Helvetica
15 Chimica Acta - Vol. 65, Fasc. 3 (1982) - Nr. 66: 661-670.
Mammals can also be utilized to synthesize several of the
resin acid derivatives of formula (I) supra, as noted in
Xenobiotica Vol. 16, No. 8 (1986): 753-767. Therefore, the
synthesis of all the resin acids and derivatives thereof
20 listed in formula (I) supra is clearly well-known.

 Preferably, the composition of the present
invention comprises the compound of formula (I) supra
wherein R_1 is H, R_2 is H, R_3 is H, R_4 is H, R_5 when present is
H, R_6 when present is H, R_7 when present is H, R_8 when
25 present is H or CH₃, R_9 when present, is H, R_{10} when present
is H, R_{11} when present is H, R_{12} is CH₃, R_{13} is CH₃ or COOH, R_{14}
is H, CH₂ or CH₃, R_{15} is H or CH₃, R_{16} when present is H/^{or CH₃}, R_{17} is
H, R_{18} is H, R_{19} is H, R_{20} when present is H, and R_{21} when
present is H.

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1 More specifically, the composition of the present
invention preferably comprises at least one compound
selected from the group consisting of 18-abietanoic acid; 13
beta-abietan-18-oic acid; 8 alpha, 13 beta-abietan-18-oic
5 acid; 9 beta, 13 beta-abietan-18-oic acid; 7-abieten-18-oic
acid; 13 beta-abiet-7-en-18-oic acid; 8-abieten-18-oic acid;
13 beta-abiet-8-en-18-oic acid; 8(14)-abieten-18-oic acid;
13 beta-abiet-8(14)-en-18-oic acid; 13-abieten-18-oic acid;
8 alpha-abiet-13-en-18-oic acid; 13(15)-abieten-18-oic acid;
10 7, 13-abietadien-18-oic acid; 8, 13-abietadien-18-oic acid;
8, 12-abietadien-18-oic acid; 8,13(15)-abietadien-18-oic
acid; 8(14), 13(15)-abietadien-18-oic acid; 13 beta-abieta-
7,9(11)-dien-18-oic acid; 8(14), 12-abietadien-18-oic acid;
8,11,13-abietatrien-18-oic acid; 6,8,11,13-abietatetraen-18-
15 oic acid; 5 beta-abieta-8,11,13-trien-18-oic acid; 18-
isopimaranoic acid; 8 alpha-isopimaran-18-oic acid; 7-
isopimaren-18-oic acid; 8-isopimaren-18-oic acid; 8(14)-
isopimaren-18-oic acid; 7,15-isopimaradien-18-oic acid;
8,15-isopimaradien-18-oic acid; 8(14),15-isopimaradien-18-
20 oic acid; 18-pimaranoic acid; 8 alpha-pimaran-18-oic acid;
8-pimaren-18-oic acid; 8(14)-pimaren-18-oic acid; 8,15-
pimaradien-18-oic acid; and 8(14),15-pimaradien-18-oic acid.

More preferably, in the compound of formula (I)
supra, R₆ is not present, i.e., there is an unsaturated
25 double bond extending from the no. 8 position on the fused
ring structure, while R₁₃ is COOH and R₁₄ is CH₂ or CH₃. In
other words, the composition of the present invention
includes at least one of the following compounds: 8,15-
isopimaradiene-oic acid; 8,15-pimaradien-18-oic acid; 7,15-
30 isopimaradiene-18-oic acid; 13 beta-abieta 7,9(11)-dien-18-

- 1 oic acid; 5 beta-abi ta-8,11,13-trien-18-oic acid; 8,12-
abietadien-18-oic acid; 7,13-abietadien-18-oic acid, and
8(14), 13(15)-abietadi n-18-oic acid.

Non-toxic, pharmaceutically acceptable acid

- 5 addition salts of these resin acids and derivatives thereof
can be prepared by conventional reactions with equivalent
amounts of organic or inorganic solutions. Exemplary acid
addition salts include hydrochloric, hydrobromic, sulfuric,
benzenesulfonic, acetic acid, fumaric acid, oxalic acid,
10 malic acid, citric acid, potassium hydroxide, and sodium
hydroxide salts of the abietic derivatives herein.

- These resin acids and derivatives thereof and/or
the pharmaceutically acceptable salts thereof are combined
with a pharmaceutically acceptable carrier for
15 administration to an individual. For example, the
derivatives can be combined with a suitable liquid carrier
for parenteral administration, including water, alcohol,
propylene glycol and to provide a suitable composition for
application. Such compositions can be injected
20 intravenously, intraperitoneally, intramuscularly or applied
topically. The compositions can also be formulated for oral
administration in liquid or solid form. Suitable carriers
for this administration route include water, alcohol, oil.

- A particular aspect of this invention comprises a
25 composition containing the resin acid or derivative and/or
salt in an "effective amount", i.e., an amount sufficient to
bring about the desired anti-tumor or tumor treating effect.
In this regard, the invention is also directed to a method
of treating tumors which comprises administering an
30 effective amount of said abietic acid derivative.

1 A preferred concentration of the resin acid or
derivative thereof and/or salt is from about 0.01 to about
0.50 mg/mg of carrier, more preferably from about 0.02 to
5 to about 0.30 mg/mg carrier and most preferably from about 0.05
administration of the resin acid or derivative thereof
and/or salt is preferably from about 100 to about 800 mg/kg
individual, more preferably from about 200 to about 700
10 mg/kg individual and most preferably from about 300 to about
600 mg/kg individual.

 The compositions of the present invention
containing one or more of the resin acids or derivatives
thereof herein are effective against a variety of tumors
including L929 cells (ATCC # CCL 1: NCTC Clone 929, clone of
15 strain L., connective tissue); S 180 cells (ATCC # TIB 66;
Sarcoma 180, sarcoma swiss webster), Ehrlich sarcoma cells
(ATCC # CCL 77: Strain E, Ehrlich-Lettre Ascites) and
against other tumor cells including, non-small cell lung
cancer, small cell lung cancer, colon cancer, CNS cancer,
20 melanoma, ovarian cancer, and renal cancer cells. At the
same time, these resin acids and derivatives thereof are
selective against only tumor cells, i.e., they do not tend
to attack normal cells. In this regard, the resin acids and
derivatives are less toxic, e.g., than the compounds
25 disclosed in Ref. Zh. Khim and Pharmaceutical Chemistry
Journal cited supra.

 The present invention will be described in greater
detail by way of the following examples:

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1

EXAMPLE 1STEP (A)OBTAINING MAJOR AND MINOR
ACTIVE FRACTIONS OF ROSIN

5

4 mg. of rosin was chromatographed using a silica HPLC column of 25 cm. length and 10 mm. internal diameter containing silica particles of about 5 microns in size. Fractions were collected, evaporated to dryness, and re-dissolved in 20 μ l of methanol, with the methanol extracts of the individual fractions each being mixed with 2ml of culture medium containing L929 tumor cells. The ability of each fraction to inhibit tumor cell growth was measured according to an assay technique based on the one designed by Flick and Gifford in J. Immunol. Meth. 68 (1984): 167-175.

10 Fig. 1 is a graph of the ability of these various rosin fractions to inhibit growth of L929 tumor cells, also illustrating ultraviolet (UV) absorbance of each fraction.

15

More specifically, Fig. 1 indicates two main peaks of biological activity among the fractions against the L929 tumor cells. The fraction of largest activity (termed "major active fraction"), exhibits maximum UV absorbance at 254 nm. The fraction of next highest activity (termed "minor active fraction"), eluted from the HPLC column approximately three minutes after the major active fraction

20 (the abscissa of the graph in Fig. 1 denotes the elution time from the HPLC column).

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SUBSTITUTE SHEET

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STEP (B)PURIFYING MAJOR ACTIVE FRACTION
OF ROSIN OBTAINED IN STEP (A)

5 The major active fraction of rosin obtained in
Step (A) was then chromatographed by HPLC using an ODS1
reverse phase column of 25 mm. length and 4.6 mm. internal
diameter containing carbon-coated silica particles.
Fractions eluting through the reverse phase column at
different times were collected, evaporated to dryness, and
10 re-dissolved in 20 μ l of methanol, with the methanol
extracts of the individual fractions each being mixed with 2
ml of culture medium containing L929 tumor cells. The
inhibiting activity of each fraction was measured as in Step
(A) above, with the results being presented in Fig. 2.
15 Fig. 2 indicates one peak of biological activity,
coincident with maximum absorbance at 254 nm. The fraction
exhibiting this peak was designated the "purified major
active fraction".

20

STEP (C)METHYL ESTERIFICATION OF PURIFIED
MAJOR ACTIVE FRACTION FROM STEP (B)

5 mg. the purified major active fraction from Step
(B) was added to 350 microliters of methanol in a tube
25 followed by the addition of 0.3 ml hexane and 0.35 ml BF_3
reagent, the tube thereafter being sealed under nitrogen.
The sealed tube was then incubated in boiling water for 40
minutes and cooled. 2 ml. hexane and 1 ml. water were added
to the opened tube, the tube then being shaken for 2
30 minutes, then allowed to stand to create phase separation

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1 into organic and aqueous layers. The organic (hexane) layer
was removed and vaporated to dryness, and then re-dissolved
in 200 μ l. of a solvent mixture of 1-5% dioxane and 1-5%
methanol in hexane and subjected to HPLC in a silica column
5 of 25 cm. length and 10 mm. internal diameter containing
silica particles of about 5 microns in size. The solvent
system was introduced into the silica column at a constant
flow rate of 3 ml/min.

At the same time, the eluted fractions were
10 subjected to ultraviolet spectroscopy to analyze the
composition thereof. It was found that the fraction
composed of resin acid methyl esters eluted almost at the
void volume within the silica column, in other words almost
immediately. The non-esterified resin acids eluted at a
15 slower rate.

The isolated resin acid methyl ester fraction was
collected, evaporated to dryness, re-dissolved in alcohol,
and then subjected to gas chromatography/mass spectroscopy,
with the analysis establishing the presence of methyl esters
20 of a fraction constituted by the following resin acids in
the following proportions in Table 1 below for the major
active fraction (methyl esterification enabled
identification of resin acids constituting the original
fraction):

25

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TABLE 1

1	<u>Resin Acid In Purified Major Active Fraction</u>	<u>% Of Total Resin Acids In Major Active Fraction</u>
	8,15-isopimaradiene-18-oate	5.2
5	8,15-pimaradien-18-oate	3.2
	7,15-isopimaradien-18-oate	9.2
	13 β -abieta-7,9(11)-dien-18-oate)	2.2
	5 β -abieta-8,11,13-trien-18-oate)	
	8,12-abietadien-18-oate	1.1
10	7,13-abietadien-18-oate	78.6
	8(14),13(15)-abietadien-18-oate	0.5

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EXAMPLE 2

1 A technical grade abietic acid fraction was
purified by normal phase HPLC, revers phase HPLC (ODS1) and
methyl esterified in accordance with the procedure outlined
5 in steps (A), (B) and (C) respectively of Example 1. Table
2 below lists the constituents and percentages in the
purified abietic acid fraction that were identified by the
methyl esterification:

TABLE 2

10	<u>Resin Acid In Purified</u> <u>Abietic Acid Fraction</u>	<u>% Of Total</u> <u>Resin Acids In</u> <u>Abietic Acid Fraction</u>
	8,15-isopimaradiene-18-oate	3.5
	7,15-isopimaradiene-18-oate	8.9
	13 β -abieta-7,9(11)-dien-18-oate)	9.7
15	5 β -abieta-8,11,13-trien-18-oate)	
	8,12-abietadien-18-oate	2.5
	7,13-abietadien-18-oate	72.5
	8(14),13(14)-abietadien-18-oate	1.8
20	unknown	1.1

25

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EXAMPLE 3

1 In accordance with the procedure outlined in Canc.
2 Res. 47: 3707-3711, female CD1 mice (obtained from Charl s
3 River Laboratories) were inoculated intraperitoneally with
4 10^6 S 180 cells in 100 microliters of phosphate buffered
5 saline (PBS). Controls received PBS only. Both tumor and
6 control mice were divided into three groups of ten mice per
7 group. Rosin and technical grade abietic acid were each
8 dissolved in ethanol at a concentration of 200 mg/ml which
9 was diluted with 10% newborn calf serum in PBS to a ratio of
10 1:20 to form respective suspensions. Untreated groups (both
11 tumor and control mice) were inoculated intraperitoneally
12 with 1 ml of vehicle alone while technical grade abietic
13 acid treated groups (both tumor and control) were
14 intraperitoneally inoculated with 1 ml (10 mg) of the rosin
15 suspension and the technical grade abietic acid treated
16 groups (both tumor and control) were intraperitoneally
17 inoculated with 1 ml (10mg) of the technical grade abietic
18 acid suspension, the doses being administered immediately,
19 and thereafter on the third, sixth and ninth days. Data
20 from animals which expired within two hours of any
21 inoculation (at zero, three, six and nine days) were
22 discarded. The survival rates of the various treated and
23 untreated groups is plotted against time in Fig. 3 with mic
24 surviving longer than ninety days being deemed cured of the
25 original intraperitoneal tumor burden. Fig. 4 records animal
26 weight (growth rate) over time of the tumor-free group, with
27 n denoting the number of mice in each treated group in Figs.
28 3 and 4.

30

35

1 Technical grade abietic acid includes the various
constituents of Tabl 2 supra as principal ingredients, in
addition to other minor amounts of associated resin acids.

5 Fig. 3 illustrates a significant difference in
survival between treated and untreated groups of mice. The
only visible side effects were increased weight (Fig. 4) and
a slight deterioration of the coat condition, notably in the
technical grade abietic acid-treated group. The notation in
Fig. 3 on $p < 0.05$ establishes that there was statistical
10 significance between the untreated mice and the rosin or
technical grade abietic acid treated mice (i.e., the
treatment has some effect). It was ascertained upon
sacrifice and dissection that the weight increase appeared
due to increased deposition of adipose tissue. The
15 asterisks in Fig. 4 denote that the difference between the
asterisked group and the control group is statistically
significant. These results clearly establish the anti-tumor
effect of rosin and technical grade abietic acid.

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EXAMPLE 4

1 Mice were treated in accordance with the procedure
described in Example 3 above with the omission of the tumor-
laden groups and the groups treated with rosin (i.e., tumor-
5 free mice were treated with either the technical grade
abietic acid or vehicle alone). On the twenty-third day
after treatment was begun, mice from each group were
sacrificed, bled, and the resulting six blood and six serum
samples therefrom subjected to respective hematological and
10 chemical analysis. On the twenty-fifth day after the start
of treatment, three technical grade abietic acid-treated
mice were subjected to necropsy and histological assessment
of selected tissues. The survival rate of the treated mice
is reported in Table 3, the hematological/chemical analysis
15 is reported in Table 4 and the histological assessment is
reported in Table 5 below.

TABLE 3

SURVIVAL OF MICE TREATED INTRAPERITONEALLY WITH
ABIETIC ACID SUSPENSION (4 doses of 430 mg abietic
acid/kg) or JUST PBS VEHICLE ALONE (CONTROL)

20	<u>Group</u>	<u>Group Size</u> <u>(number of mice)</u>	<u>Survivors at 23</u> <u>(number of mice)</u>
	Control	30	22*
	Treated	30	21*

25 *p>0.05 (chi-squared test)

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TABLE 4

BLOOD HEMATOLOGY AND CHEMISTRY OF MICE TREATED
INTRAPERITONEALLY WITH ABIETIC ACID SUSPENSION
(4 doses of 430 mg/kg) OR JUST VEHICLE ALONE (CONTROL)

	Parameter	Observed Group Control	Mean +/-SEM (n) Treated	Units	Students t
5	Red Blood Cell Count	8.84±0.16(6)	8.57±0.26(6)	10 ⁶ /ul	NS
10	White Blood Cell Count	3.53±0.25(6)	3.50±0.39(6)	10 ³ /ul	NS
	Hematocrit	48.0±0.86(6)	45.4±1.48(6)	%	NS
	Hemoglobin (HB)	14.6±0.24(6)	13.9±0.37(6)	g/100ml	NS
15	Mean Cell Volume	54.3±0.87(6)	53.1±1.99(6)	fl	NS
	Mean Cell Hemoglobin	16.5±0.16(6)	16.3±0.27(6)	pg	NS
20	Mean Corpuscular HB Conc.	30.4±0.54(6)	30.8±0.98(6)	g/100ml	NS
	Segmented Neutrophils	7.16±0.98(6)	5.33±1.45(6)	%	NS
	Band Neutrophils	0(6)	0(6)	%	NS
25	Lymphocytes	91.8±0.95(6)	93.3±1.69(6)	%	NS
	Monocytes	1.0±0.63(6)	1.3±0.49(6)	%	NS
	Eosinophils	0(6)	0(6)	%	NS
	Basophils	0(6)	0(6)	%	NS
30	Nucleated RBC	0(6)	0(6)	/100 WBC	NS

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TABLE 4 (CONTINUED)

BLOOD HEMATOLOGY AND CHEMISTRY OF MICE TREATED
INTRAPERITONEALLY WITH ABIETIC ACID SUSPENSION
(4 doses of 430 mg/kg) OR JUST VEHICLE ALONE (CONTROL)

5	Parameter	Observed Group Mean +/-SEM (n)		Units	Students t
		Control	Treated		
	Platelets	Adequate(6/6)	Adequate(6/6)		
	RBC Morphology	Normal (6/6)	Normal (6/6)		
	Glucose	162±5.8 (5)	152±6.7 (6)	mg/100ml	NS
10	BUN	14.6±0.51 (5)	16.0±0.89 (6)	mg/100ml	NS
	Creatinine	0.40±0.03 (6)	0.38±0.02 (6)	mg/100ml	NS
	Total Protein	5.54±0.10 (5)	5.43±0.10 (6)	g/100ml	NS
	Albumin	3.84±0.04 (5)	3.61±0.11 (6)	g/100ml	p<0.05*
15	Calcium	7.32±0.21 (5)	7.90±0.36 (6)	mg/100ml	NS
	Inorganic Phosphorus	8.24±0.17 (5)	9.83±0.41 (6)	mg/100ml	p<0.05
	Alkaline Phosphatase	121±11.7 (5)	149±14.6 (6)	U/1	NS
20	AST (SGOT)	303±29.7 (5)	341±44.9 (5)	U/1	NS
	ALT (SGPT)	74.0±6.9 (5)	66.8±3.9 (5)	U/1	NS
	LDH	1329±129 (5)	1496±127 (5)	U/1	NS
25	Cholesterol	110±5.2 (5)	119±6.0 (5)	mg/100ml	NS
	Total Bilirubin	0.36±0.019 (5)	0.38±0.017 (5)	mg/100ml	NS
	Amylase	4988±366 (5)	5704±170 (5)	U/1	NS
30	Sodium	138.0±1.3 (5)	141.3±0.99 (6)	meq/1	NS

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TABLE 4 (CONTINUED)

BLOOD HEMATOLOGY AND CHEMISTRY OF MICE TREATED
INTRAPERITONEALLY WITH ABIETIC ACID SUSPENSION
(4 doses of 430 mg/kg) OR JUST VEHICLE ALONE (CONTROL)

5	<u>Parameter</u>	<u>Observed Group Mean +/-SEM (n)</u>		<u>Units</u>	<u>Students t</u>
		<u>Control</u>	<u>Treated</u>		
	Chloride	87.4±2.36(5)	88.0±0.97(6)	meq/l	NS
	Globulin	1.70±0.11(5)	1.82±0.031(6)	g/100ml	NS
10	Albumin/ Globulin Ratio	2.30±0.154(5)	2.00±0.078(6)		NS
	BUN/Creatinine Ratio	374±33.0(5)	421±26.9(6)		NS

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Mann - Whitney ranking test (corrected for ties).

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TABLE 5

HISTOLOGICAL ASSESSMENT OF MICE TREATED
INTRAPERITONEALLY WITH 4 DOSES OF 430 MG/KG OF ABIETIC
ACID SUSPENSION

5	<u>Mouse No:</u>	<u>1</u>	<u>2</u>	<u>3</u>
	<u>Gross necropsy</u>			
	Heart	-	-	-
	Lung	-	E	-
	Liver	A	-	-
10	Spleen	B	B	B
	Kidney	-	-	-
	Eyes	-	-	-
	Other	CD	-	F
	<u>Histological assessment</u>			
	Heart	-	-	-
15	Liver	G	J	-
	Spleen	H	H	H
	Kidney	-	-	K
	Stomach	I	-	-
	Lung	-	-	-
	Pancreas	-	-	L
20	<u>Legend</u>			
	- = No significant findings.			
	A = Liver: The liver adjacent to the spleen had a slightly pale firm area.			
25	B = Spleen: The splenic capsule had a mottled opaque blue appearance.			
	C = Stomach: The serosal surface appeared to have some diverticula, but no diverticula were observed once the stomach was opened.			
30	D = Small intestine: The small intestine was adhered to the peritoneum.			

- 1 E = Lung: The right cranial lobe had a dark red wedge shaped focus which disappeared once the lung was inflated with formalin.
- F = Pancreas: The pancreas appeared to be slightly enlarged.
- 5 G = Liver: The hepatic capsule was mildly thickened with fibrous tissue and a modicum of mononuclear cells and neutrophils. Rare small foci of several neutrophils with or without several mononuclear cells were in the parenchyma. A modicum of neutrophils was also surrounding one bile duct.
- 10 H = Spleen: The splenic capsule was mildly thickened with fibrous tissue and moderate numbers of mononuclear cells and neutrophils and less amounts of eosinophils. Neutrophils and eosinophils were also present in the parenchyma beneath the capsule. There was also mild lymphoid hyperplasia.
- 15 I = Stomach: There was a relatively small focus of fibrous tissue and rare eosinophils on the serosa of the stomach. Several foci of fibrous tissue with mononuclear cells, neutrophils and eosinophils were in the abdominal fat in close proximity to the stomach. A modicum of neutrophils was also in a neighboring abdominal lymph node.
- 20 J = Liver: There were rare small foci of several mononuclear cells and a few neutrophils in the parenchyma.
- K = Kidney: Cortical cyst.
- 25 L = Pancreas: There was a modest amount of fibrous tissue with a few mononuclear cells and neutrophils adjacent to the pancreas.

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SUBSTITUTE SHEET

1 Final Diagnosis

1. Mild multifocal chronic-active perisplenitis. (Mic
Nos. 1,2 & 3)
2. Mild multifocal chronic-active perihepatitis. (Mouse
No. 1)
3. Minimal to mild multifocal chronic-active peritonitis.
(Mice Nos. 1 & 2).

Toxicity

There was no conclusive evidence of toxicity but the perisplenitis, perihepatitis and peritonitis were likely secondary to irritation from the test compound. In addition, the presence of the eosinophils in the lesions might be indicative of a possible hypersensitivity (allergic) response.

The data in Table 3 indicate no detectable difference in frequency of unexpected deaths between the two groups of mice while the Table 4 data show no detectable difference between these two groups of mice except for a minor reduction in albumin and a minor increment in inorganic phosphorous in the abietic acid treated mice. In particular, white blood cell count and platelet assessment remained unchanged by treatment with abietic acid. The data in Table 5 shows that there was no conclusive histological evidence of toxicity. The observed perisplenitis, perihepatitis and peritonitis were probably due to secondary irritation from the injected substances.

Table 3 indicates that the intraperitoneal administration of vehicle alone also resulted in a mortality rate of approximately 27%. Most of the deaths for both technical grade abietic acid-treated mice and just the

1 vehicle-treated mic occurred after the fourth injection.
This mortality rate is therefore possibly due to the
alcoholic content of the vehicle itself. Nevertheless,
there is clearly no demonstrable difference in the mortality
5 rate between the control and treated groups of mice
establishing that the dose of 430 mg/kg of technical grade
abietic acid (administered four times) exhibits no
detectable lethal effect over this documented time period.
In contrast, the alkylating resin acid derivatives of Table
10 I of the Pharmaceutical Chemistry Journal publication cited
above, when administered intraperitoneally in starch paste,
exhibited an LD₅₀ ranging from 30-500 mg/kg, giving a median
value of 250 mg/kg. It is therefore clear that technical
grade abietic acid has significantly lower acute
15 intraperitoneal toxicity than the alkylating derivatives of
resin acids disclosed in the Pharmaceutical Chemistry
Journal publication.

Table 4 shows that there is little significant
difference between the technical grade abietic acid-treated
20 group and the control group with respect to blood hematology
and chemistry. There is no demonstrable effect on white
blood cell count or platelet status, even after four doses
which each exceeded the LD₅₀ of alkylating agents derived
from resin acids as disclosed in the Pharmaceutical
25 Chemistry Journal publication. Alkylating agents as a class
of anti-tumor compounds are known to affect rapidly
proliferating normal tissue resulting in lowered white blood
cells and platelet counts. The white cell count nadir for
most alkylating agents is between 7 and 21 days and is often
30 used as the defining limit of clinical treatment (Cline and

- 1 Haskell, "Drugs Used in Cancer Chemotherapy" Third dition,
W.B. Saunders Co. (1980), pages 31-44).

Table 5 shows that histological valuation of
three animals treated with technical grade abietic acid
5 revealed no evidence of toxicity but only minor secondary
irritations. Therefore, technical grade abietic acid has
significantly lower toxicity than alkylating agents from
resin acids as disclosed in The Pharmaceutical Chemistry
Journal publication when administered intraperitoneally.

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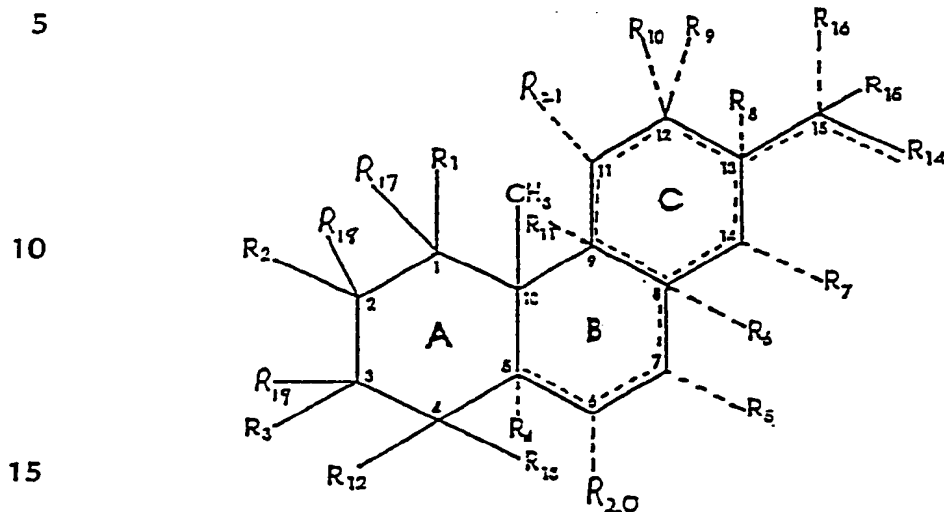
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SUBSTITUTE SHEET

1 WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a compound of the following formula:



wherein

20 R_1 is H or OH,
 R_2 is H, OH or OCOCH_3 ,
 R_3 is H, OCH_3 or $\text{CH}(\text{COOCH}_3)_2$,
 R_4 , when present, is H or OCH_3 ,
 R_5 , when present, is H, $=\text{O}$, CH_2OH or $\text{CH}_2\text{OCOCH}_3$,
 R_6 , when present, is H, OH, CHO or CHS_2 ,
 R_7 , when present, is H, $=\text{O}$, Cl, OH, OCH_3 , COOH,
25 OCOCH_3 , CH_2OH or $\text{CH}_2\text{OCOCH}_3$,
 R_8 , when present, is H or OH,
 R_9 , when present, is H or CH_3 ,

- 1 R_{10} , when present, is H, Cl, CH_3 , CH_2Cl , CH_2CN ,
 CH_2OH , $COOCH_3$, CH_2COOH , $CH_2CH_2NH_2$, CH_2OCOCH_3 ,
 CH_2CH_2NCO , CH_2COOCH_3 , $CH_2CH_2NHCONHC_6H_5$,
 $CH_2O(CH_2CCH_3OH)_xH$ where x is a positive
5 integer, $CH_2CH_2NHCONHC_6H_{11}$, $CH_2OCO(CH_2)_4COOC_2H_4OH$
or $CH_2CH_2NH_2 \cdot HOOC_6H_3(NO_2)_2$,
 R_{11} , when present, is H or OH,
 R_{12} is H, CH_3 or $COOCH_3$,
 R_{13} is CN, CH_3 , $COCl$, $COOH$, $CONH_2$, $COCH_3$, CH_2OH ,
10 CH_2NH_2 , $COOCH_3$, CH_2NCO , CH_2OCOOH , CH_2OCOCH_3 ,
 $CH_2O(CHCHO)_xH$ where x is a positive integer,
 $CH_2NHCONHC_6H_5$, $CH_2NHCONHC_6H_{11}$, CH_2O
 $(CH_2CCH_3HO)_xH$ where x is a positive integer,
 $CH_2NH_2 \cdot HOOC_6H_3(NO_2)_2$ or
15 $CH_2OCO(CH_2)_4COOC_2H_4OH$,
 R_{14} is H, CH_3 , CH_2 , $COOH$, CH_2OH or $COOCH_3$,
 R_{15} is H, OH, $OCOOH$, $\overset{H}{/}$,
 R_{16} , when present, is $\overset{H}{/}CH_3$ or CH_2OH ,
 R_{17} is H or OH,
20 R_{18} is H, OH or $OCOOH$,
 R_{19} is H, OCH_3 or $CH(COOCH_3)_2$,
 R_{20} , when present, is H, = O, CH_2OH or CH_2OCOCH_3 ,
and
 R_{21} , when present, is H or CH_3 ,
25 and the pharmaceutically acceptable salts thereof
together with a pharmaceutically acceptable carrier
therefor.

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INSTITUTE SHEET

1 2. The composition of Claim 1, wherein
R₁ = H; R₂ = H; R₃ = H; R₄ = H; R₅ when present = H; R₆ when
present = H; R₇ when present = H; R₈ when present = H or CH₃;
R₉ when present = H; R₁₀ when present = H; R₁₁ when present =
5 H; R₁₂ = CH₃; R₁₃ is CH₃ or COOH; R₁₄ is H, CH₂ or CH₃; R₁₅ = H or
CH₃, R₁₆ when present = H; R₁₇ = H; R₁₈ = H; R₁₉ = H; R₂₀ when
present = H; and R₂₁ when present = H.

3. The composition of Claim 2, wherein said
compound is at least one member selected from the group
10 consisting of 18-abietanoic acid; 13 beta-abietan-18-oic
acid; 8 alpha, 13 beta-abietan-18-oic acid; 9 beta, 13 beta-
abietan-18-oic acid; 7-abieten-18-oic acid; 13 beta-abiet-7-
en-18-oic acid; 8-abieten-18-oic acid; 13 beta-abiet-8-en-
18-oic acid; 8(14)-abieten-18-oic acid; 13 beta-abiet-8(14)-
15 en-18-oic acid; 13-abieten-18-oic acid; 8 alpha-abiet-13-en-
18-oic acid; 13(15)-abieten-18-oic acid; 7, 13-abietadien-
18-oic acid; 8, 13-abietadien-18-oic acid; 8, 12-abietadien-
18-oic acid; 8,13(15)-abietadien-18-oic acid; 8(14), 13(15)-
abietadien-18-oic acid; 13 beta-abieta-7,9(11)-dien-18-oic
20 acid; 8(14), 12-abietadien-18-oic acid; 8,11,13-abietatrien-
18-oic acid; 6,8,11,13-abietatetraen-18-oic acid; 5 beta-
abieta-8,11,13-trien-18-oic acid; 18-isopimaranoic acid; 8
alpha-isopimaran-18-oic acid; 7-isopimaren-18-oic acid; 8-
isopimaren-18-oic acid; 8(14)-isopimaren-18-oic acid; 7,15-
25 isopimaradien-18-oic acid; 8,15-isopimaradien-18-oic acid;
8(14),15-isopimaradien-18-oic acid; 18-pimaranoic acid; 8
alpha-pimaran-18-oic acid; 8-pimaren-18-oic acid; 8(14)-
pimaren-18-oic acid; 8,15-pimaradien-18-oic acid; and
8(14),15-pimaradien-18-oic acid.

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4. The composition of Claim 2, wherein R_6 is not present, there being unsaturation present at the n . 8 position on the fused ring structure between rings B and C, $R_{13} = \text{COOH}$, and $R_{14} = \text{CH}_2$ or CH_3 .

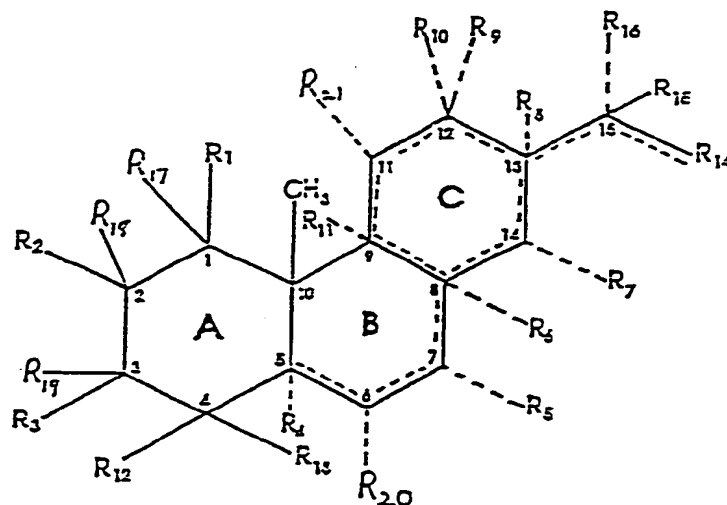
5. The composition of Claim 4, wherein said compound is at least one member selected from the group consisting of 8,15-isopimaradiene-oic acid; 8,15-pimaradien-18-oic acid; 7,15-isopimaradiene-18-oic acid; 13 beta-abieta 7,9(11)-dien-18-oic acid; 5 beta-abieta-8,11,13-trien-18-oic acid; 8,12-abietadien-18-oic acid; 7,13-abietadien-18-oic acid, and 8(14), 13(15)-abietadien-18-oic acid.

6. The composition of Claim 1 wherein the compound is present at a level of from about 0.01 to about 0.30 mg/mg carrier.

7. The composition of Claim 6 wherein the compound is present at a level of from about 0.02 to about 0.30 mg/mg carrier.

8. The composition of Claim 7 wherein the compound is present at a level of from about 0.05 to about 0.20 mg/mg carrier.

9. A method for treating a tumor comprising administering to an individual, a tumor-treating effective amount of at least one compound of the formula:



- 1 wher in R_1 is H or OH,
 R_2 is H, OH or OCOOH,
 R_3 is H, OCH₃ or CH(COOCH₃)₂,
 R_4 , when present, is H or OCH₃,
5 R_5 , when present, is H, =O, CH₂OH or CH₂OCOCH₃,
 R_6 , when present, is H, OH, CHO or CHS₂,
 R_7 , when present, is H, = O, Cl, OH, OCH₃, COOH,
OCOOH, CH₂OH or CH₂OCOCH₃,
 R_8 , when present, is H/^{CH₃} or OH,
10 R_9 , when present, is H or CH₃,
 R_{10} , when present, is H, Cl, CH₃, CH₂Cl, CH₂CN,
CH₂OH, COOCH₃, CH₂COOH, CH₂CH₂NH₂, CH₂OCOCH₃,
CH₂CH₂NCO, CH₂COOCH₃, CH₂CH₂NHCONHC₆H₅,
CH₂O(CH₂CCH₃OH)_x where x is a positive
15 integer, CH₂CH₂NHCONHC₆H₁₁, CH₂OCO(CH₂)₄COOC₂H₄OH
or CH₂CH₂NH₂·HOOC₆H₃(NO₂)₂,
 R_{11} , when present, is H or OH,
 R_{12} is H, CH₃ or COOCH₃,
 R_{13} is CN, CH₃, COCl, COOH, CONH₂, COCH₃, CH₂OH,
20 CH₂NH₂, COOCH₃, CH₂NCO, CH₂OCOOH, CH₂OCOCH₃,
CH₂O(CHCHO)_x where x is a positive integer,
CH₂NHCONHC₆H₅, CH₂NHCONHC₆H₁₁, CH₂O
(CH₂CCH₃HO)_x where x is a positive integer,
CH₂NH₂·HOCC₆H₃(NO₂)₂ or CH₂OCO(CH₂)₄COOC₂H₄OH,
25 R_{14} is H, CH₃, CH₂, COOH, CH₂OH or COOCH₃,
 R_{15} is H, OH, OCOOH,
 R_{16} , when present, is ^H/CH₃ or CH₂OH,
 R_{17} is H or OH,
 R_{18} is H, OH or OCOOH,
30 R_{19} is H, OCH₃ or CH(COOCH₃)₂,

1 R_{20} , when present, is H, = O, CH_2OH or CH_2OCOCH_3 ,
and
 R_{21} , when present, is H or CH_3 ,
and the pharmaceutically acceptable salts thereof.

5 10. The method of Claim 9, wherein

$R_1 = H$; $R_2 = H$; $R_3 = H$; $R_4 = H$; R_5 when present = H; R_6 when
present = H; R_7 when present = H; R_8 when present = H or CH_3 ;
 R_9 when present = H; R_{10} when present = H; R_{11} when present =
H; $R_{12} = CH_3$; R_{13} is CH_3 or $COOH$; R_{14} is H, CH_2 or CH_3 ; $R_{15} = H$ or
10 CH_3 ; R_{16} when present = H; $R_{17} = H$; $R_{18} = H$; $R_{19} = H$; R_{20} when
present = H; and R_{21} when present = H.

11. The method of Claim 10, wherein said
compound is at least one member selected from the group
consisting of 18-abietanoic acid; 13 beta-abietan-18-oic
15 acid; 8 alpha, 13 beta-abieten-18-oic acid; 9 beta, 13 beta-
abietan-18-oic acid; 7-abieten-18-oic acid; 13 beta-abiet-7-
en-18-oic acid; 8-abietan-18-oic acid; 13 beta-abiet-8-en-
18-oic acid; 8(14)-abieten-18-oic acid; 13 beta-abiet-8(14)-
en-18-oic acid; 13-abieten-18-oic acid; 8 alpha-abiet-13-en-
20 18-oic acid; 13(15)-abieten-18-oic acid; 7, 13-abietadien-
18-oic acid; 8, 13-abietadien-18-oic acid; 8, 12-abietadien-
18-oic acid; 8,13(15)-abietadien-18-oic acid; 8(14), 13(15)-
abietadien-18-oic acid; 13 beta-abieta-7,9(11)-dien-18-oic
acid; 8(14), 12-abietadien-18-oic acid; 8,11,13-abietatrien-
25 18-oic acid; 6,8,11,13-abietatetraen-18-oic acid; 5 beta-
abieta-8,11,13-trien-18-oic acid; 18-isopimaranoic acid; 8
alpha-isopimaran-18-oic acid; 7-isopimaren-18-oic acid; 8-
isopimaren-18-oic acid; 8(14)-isopimaren-18-oic acid; 7,15-
isopimaradien-18-oic acid; 8,15-isopimaradien-18-oic acid;

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1 8(14),15-isopimaradien-18-oic acid; 18-pimaranoic acid; 8
alpha-pimaran-18-oic acid; 8-pimaren-18-oic acid; 8(14)-
pimaren-18-oic acid; 8,15-pimaradien-18-oic acid; and
8(14),15-pimaradien-18-oic acid.

5 12. The method of Claim 11, wherein R_6 is
not present, there being unsaturation present at the no. 8
position on the fused ring structure between rings B and C,
 $R_{13} = \text{COOH}$, and $R_{14} = \text{CH}_2$ or CH_3 .

10 13. The method of Claim 12, wherein said
compound is at least one member selected from the group
consisting of 8,15-isopimaradien-18-oic acid; 8,15-
pimaradien-18-oic acid; 7,15-isopimaradiene-18-oic acid; 13
beta-abieta 7,9(11)-dien-18-oic acid; 5 beta-abieta-8,11,13-
trien-18-oic acid; 8,12-abietadien-18-oic acid; 7,13-
15 abietadien-18-oic acid, and 8(14), 13(15)-abietadien-18-oic
acid.

14. The method of Claim 9, wherein the compound
is administered at a daily dosage of about 100 to about 800
mg/kg individual.

20 15. The method of Claim 14, wherein the compound
is administered at a daily dosage of about 200 to about 700
mg/kg individual.

25 16. The method of Claim 15, wherein the compound
is administered at a daily dosage of about 300 to about 600
mg/kg individual.

30 17. The method of Claim 9, wherein the compound
is effective against tumors selected from the group
consisting of non-small cell lung cancer, small cell lung
cancer, colon cancer, CNS cancer, melanoma, ovarian cancer
and renal cancer.

1 18. The method of Claim 9, wherein said compound
is effective against tumors selected from the group
consisting of L929 cells, S180 cells, and Ehrlich sarcoma
cells.

5 19. The composition of Claim 5, wherein said
compound is 7,13-abietadien-18-oic acid.

20. The method of Claim 13, wherein said compound
is 7,13-abietadien-18-oic acid.

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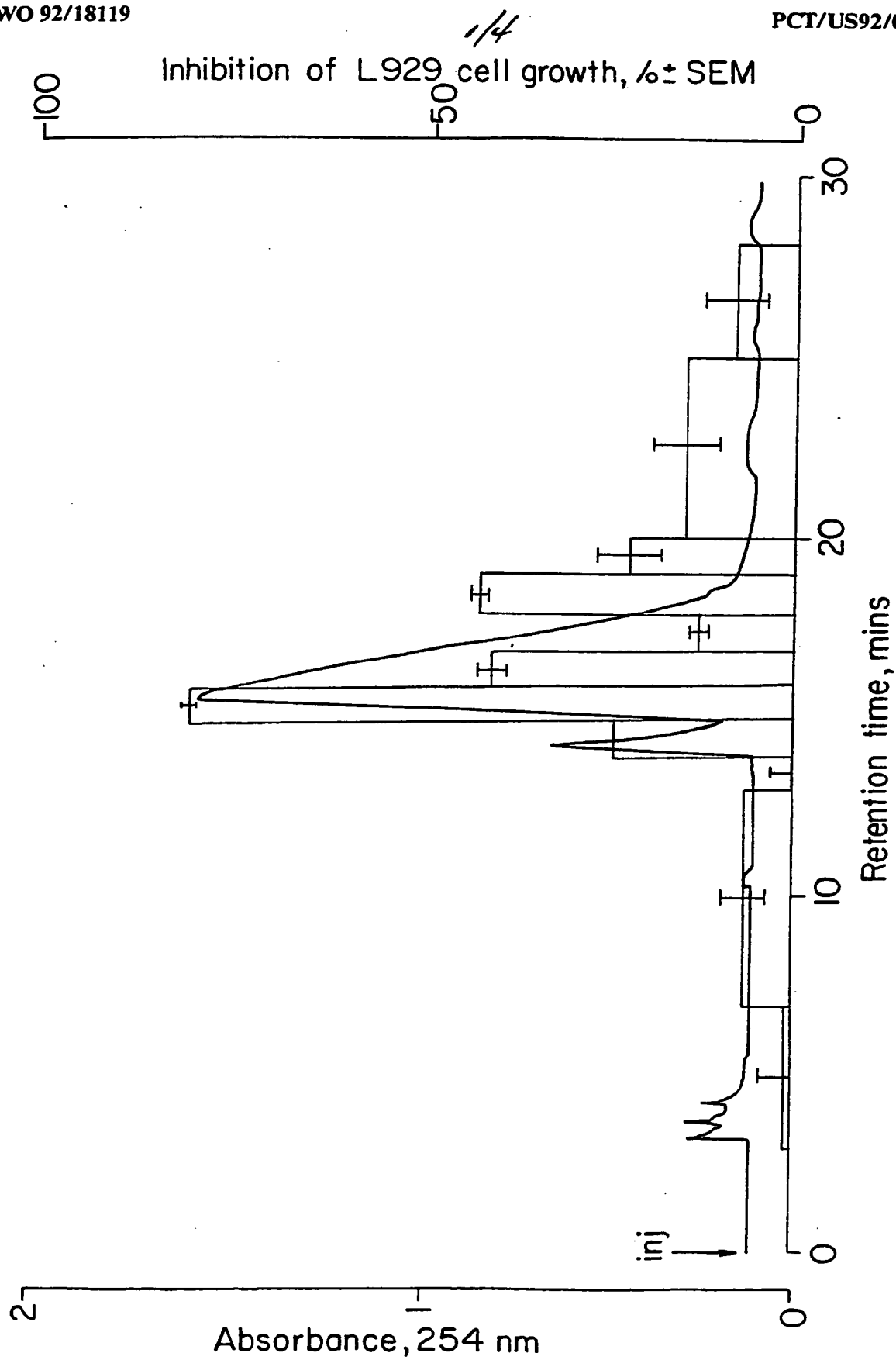


FIG. 1

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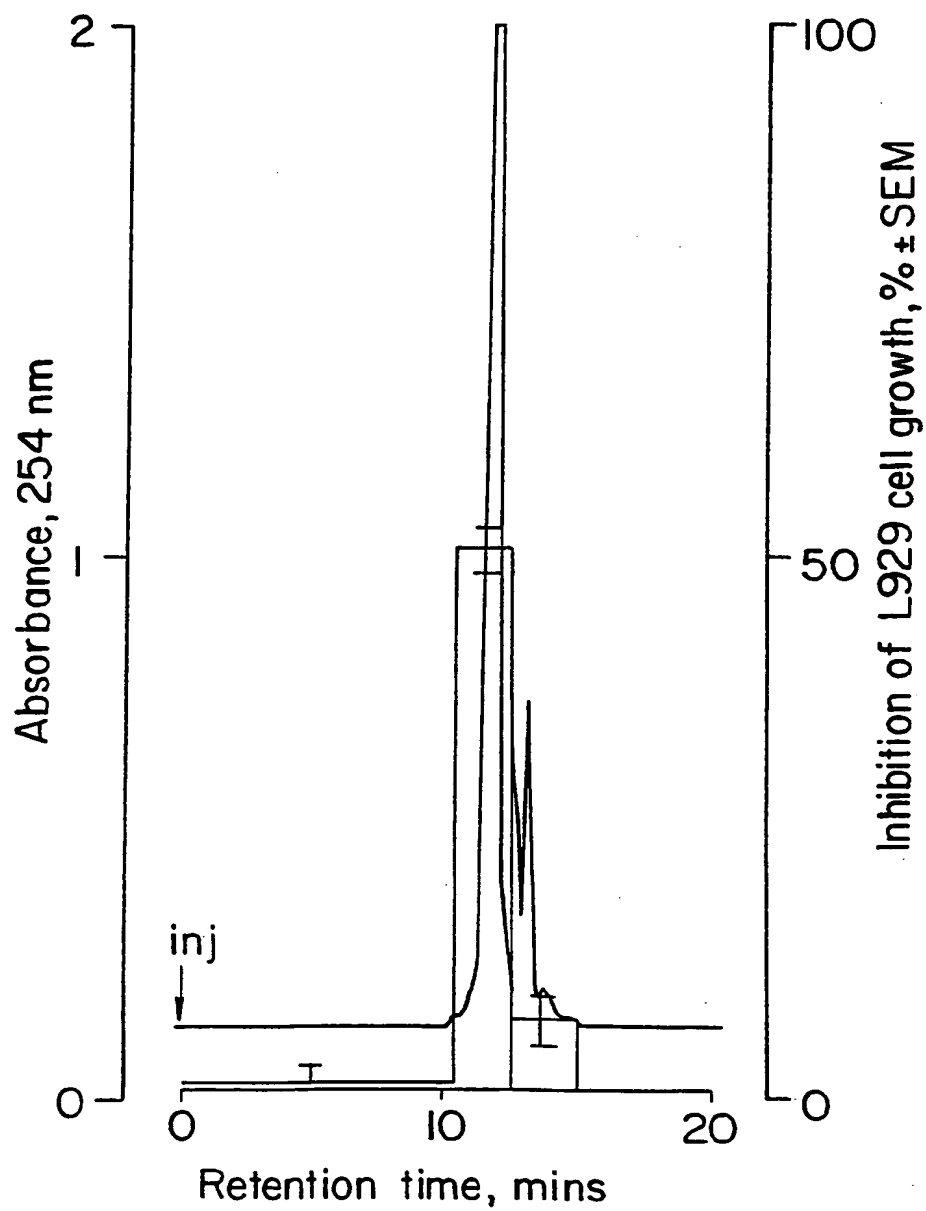


FIG. 2

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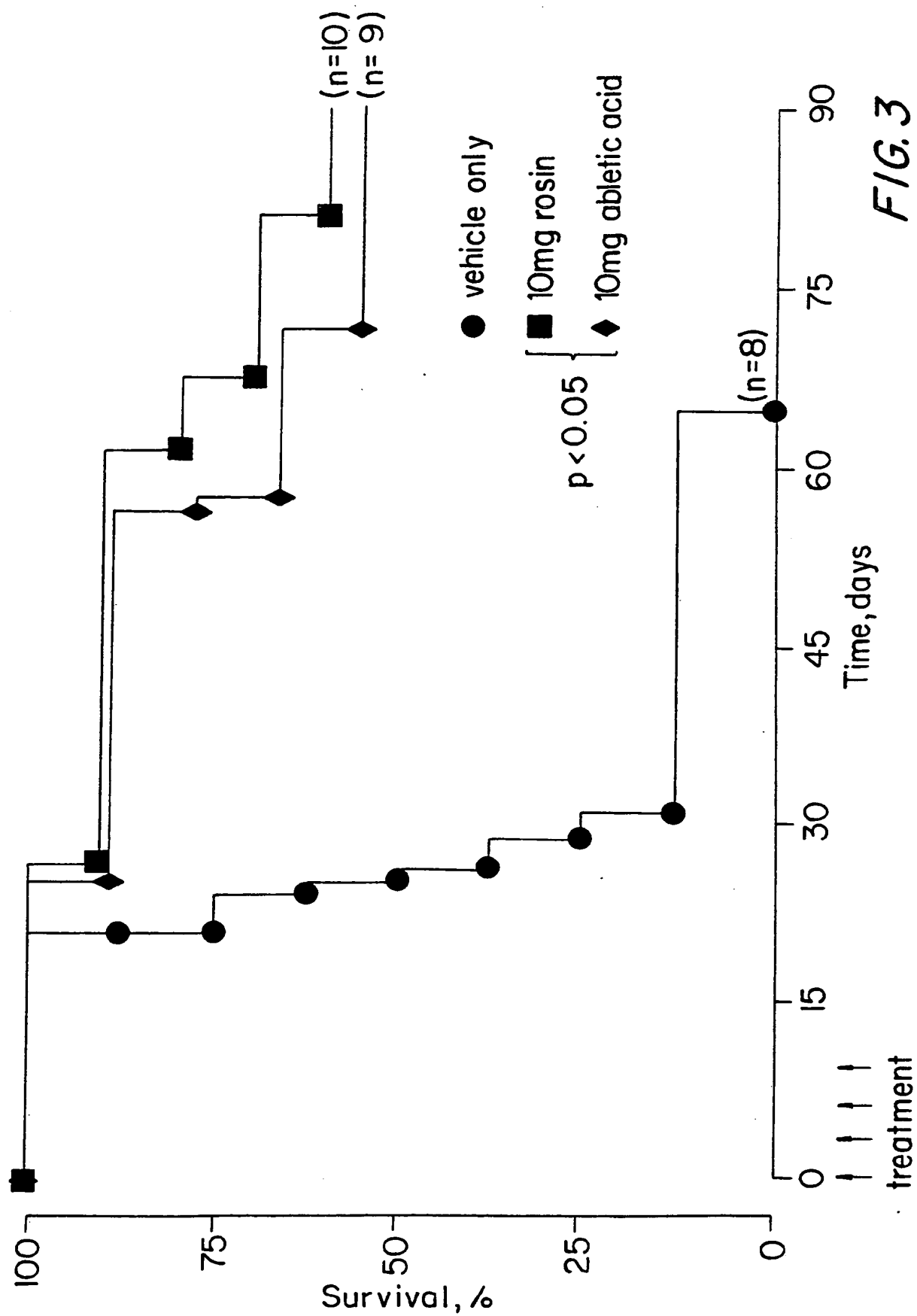
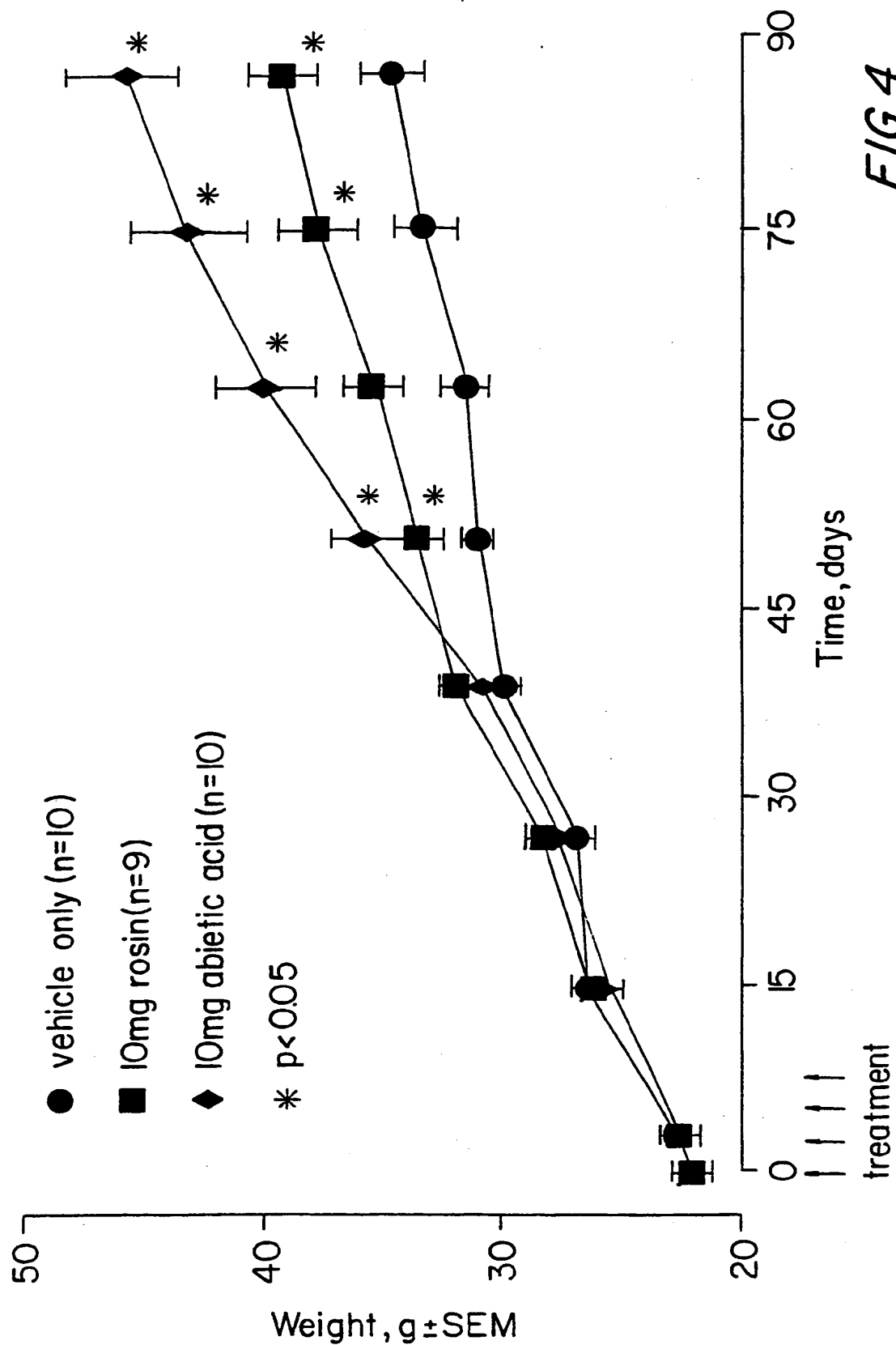


FIG. 3



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/03209

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/508,519,521,529,533,534,538,541,557,561,562,564,569,595,596,
654,659,676,677,680,691,695,706,716,719,729,732,753,755,765,766

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A/, TIANPEI ET AL, PHARMACOLOGICAL STUDY OF ANTITHROMBOTIC ACTION OF ABIETIC ACID, <u>J. OF TRADITIONAL CHINESE MEDICINE</u> , S(2); 115-118, 1985 ENTIRE REFERENCE	1-8 AND 19

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A document defining the general state of the art which is not considered to be part of particular relevance	*X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E earlier document published on or after the international filing date	*Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z document member of the same patent family
*O document referring to an oral disclosure, use, exhibition or other means	
*P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

30 JULY 1992

Date of mailing of the international search report

16 SEP 1992

 Name and mailing address of the ISA/
 Commissioner of Patents and Trademarks
 Box PCT
 Washington, D.C. 20231

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/03209

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

A61K 31/215; 31/275; 31/235; 31/24; 31/19; 31/195; 31/17; 31/135; 31/13; 31/12; 31/115; 31/095; 31/075; 31/045;
31/05; 31/03; 31/025; 31/015

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

514/508,519,521,529,533,534,538,541,557,561,562,564,569,595,596,
654,659,676,677,680,691,695,706,716,719,729,732,753,755,765,766